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Bacterial Swarming: A Re-examination of Cell-Movement Patterns

Review

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Many bacteria simultaneously grow and spread rapidly over a surface that supplies them with nutrient. Called 'swarming', this pattern of movement directs new cells to the edge of the colony. Swarming reduces competition between cells for nutrients, speeding growth. Behind the swarm edge, where the cell density is higher, growth is limited by transport of nutrient from the subsurface to the overlying cells. Despite years of study, the choreography of swarm cell movement, the bacterial equivalent of dancing toward an exit in a very dense crowd of moving bodies, remains a mystery. Swarming can be propelled by rotating flagella, and either by pulling with type IV pili or by pushing with the secretion of slime. By identifying patterns of movement that are common to swarms making use of different engines, a model of swarm choreography can be proposed.

Introduction

Interactions between different types of cells facilitate symbiotic or pathogenic associations of bacteria with animals or plants [1]. Other interactions between cells of the same species organize developmental processes, like sporulation [2]. This review is an examination of a single type of interaction between cells relating to their movement — swarming. Swarming is found within bacterial colonies that are simultaneously growing and spreading over a surface from which they absorb water and nutrient, such as agar or eukaryotic cells in a tissue. Other views of swarming have been expressed in several recent reviews [3–5].

The Bacteriodetes — a group of bacteria that includes Cytophaga, Flavobacteria and Bacterioides — and the Myxobacteria, all lacking flagella, form flat spreading colonies on agar that Stanier [6,7] described as swarms. All these bacteria are long flexible rods that spread rapidly on moist agar. Henrichsen [8] recognized their ability to swarm in the broad sense, but he chose, arbitrarily in my view, to limit use of the term 'swarming' to elongated bacteria that swim in a surface film of liquid by rotating their flagella. The Bacteriodetes and the Myxobacteria, which lack flagella, were said to glide or to twitch, but not to swarm [8]. Unfortunately Henrichsen's narrow definitions have dominated the literature on swarming and bacterial motility in general [3,9]. Those definitions have tended to hide similarities between the swarming movements of flagellated and non-flagellated bacteria.

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One aim of this review is to suggest how the behavior of non-flagellates clarifies swarm movements.

Consider *Cytophaga hutchinsonii*, a cellulolytic member of the Bacteriodetes. Henrichsen described cytophaga spreading as 'gliding', which speaks only to the movement of individual cells and says nothing about group behavior. Moving as a group, many *C. hutchinsonii* cells swarm over a patch of cellulose as they devour it to support their growth [10]. The rod-shaped, Gram-negative *C. hutchinsonii* have neither flagella on their bodies [11] nor flagellar genes in their genome [10]. Moreover, *C. hutchinsonii* resembles *Flavobacterium johnsonii*, another bacteriodete, in those respects. Only, *F. johnsonii* swarms as it digests chitin, like cellulose, a common particulate in soil [12,13]. Of the more than 40 different species of Myxobacteria none has flagella and none is able to swim. Their swarming has been appreciated ever since they were recognized as bacteria, not fungi [14]. Using a camera lucida, Thaxter accurately sketched many individual cells within their swarms [15]. Kuhlwein and Reichenbach [16] made time-lapse movies showing how cells moved within swarms. Like the Bacteriodetes, the Myxobacteria are long, thin flexible rods that glide on surfaces in the direction of their long axis.

One reason to include the Bacteriodetes and the Myxobacteria in the list of swarmers is that both the structure of swarm cells (all are long, flexible rods) and the arrangement of cells within the resulting swarms are similar to flagellated swarmers. Panels A and B in Figure 1 show swarms of a representative flagellate and a non-flagellate; both swarms have the characteristic fried-egg profile, the cross-section of which is sketched in Figure 1C. Neither swarm has the sharp edge or heaped center of a typical colony on agar that is not spreading. The similarities between a swarm produced by cells rotating flagella and one produced by cells lacking flagella suggest that the movement strategy of swarming transcends the mechanics of the engines that happen to be employed. Figure 1C emphasizes that the edge of the swarm is only one cell thick. The cells of zone 2 in Figure 1C have direct access to oxygen from above, to soluble nutrients from below, and they should be able to eliminate wastes, like ammonia, efficiently. Swarming appears to depend on a style or strategy of movement (the choreography) that allows cells to move in a generally radial direction and to slip past each other. To make the case for a general choreography, it is necessary to compare the three swarm-propulsive engines currently known and the way each is regulated — the flagella, type IV pili and slime secretion.

Swarming with and without Flagella

Rotating Screw-Like Flagella

The spreading of *Salmonella typhimurium* over solid surfaces by swarming requires cell elongation and a concomitant increased production of flagella. For

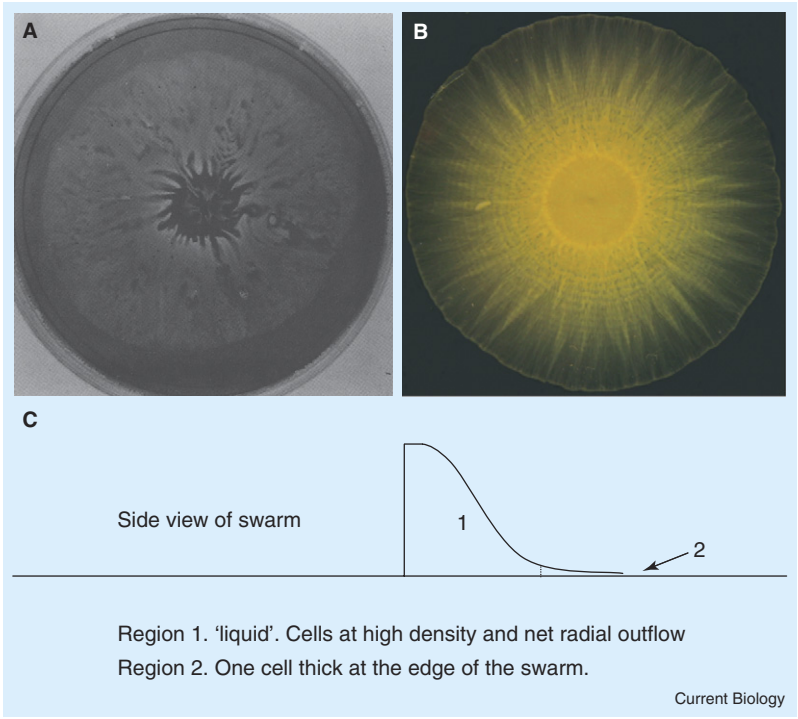

Figure 1. Swarming bacteria.

(A) Swarm of *Serratia marcesens* that can spread several millimeters per hour at 30°C. (B) Swarm of *M. xanthus*, strain DK1622, on agar. A total of 2.5×10^7 cells were spotted on a 0.5 × CTT, 0.4% agar plate and the photo was taken by Lotte Jelsbak after several days of incubation at 32°C. The center of the swarm has several layers of cells. At the edge of the swarm, a single layer of single cells and lateral clusters of cells are spreading outward. The rings and spokes are areas of high cell density. (C) Diagram of a cross section through a swarm of *M. xanthus*, such as the one shown in B. (A) modified with permission from [88]; (B) reproduced with permission from [6].

the purpose of swarming, growing cells elongate with flagella emerging at apparently random points on their surface [17]. Flagellar filaments are long, rigid, left-handed helices that do hydrodynamic work when the helix rotates and exerts force on the suspending medium [17]. When all flagella on a cell rotate in the counter-clockwise sense, and because the helices are left-handed, they can bundle and rotate together. On the other hand, if all the filaments rotate clockwise, the bundles would fly apart and the cell body could turn [17].

Switching back and forth between clockwise and counter-clockwise rotation is the function of the binding of activated CheY (CheY~P) to the switch protein FliM [18,19]. CheY is activated in *S. typhimurium* and many other Gram-negative bacteria by a set of chemosensory proteins that include a methyl-accepting chemosensory protein (the MCP), and proteins CheR, CheB, CheY and CheZ, which together support swimming chemotaxis in undifferentiated (swimming) cells. Mutations in the *Che* genes show that they are also necessary for swarming, along with several other cell surface components that facilitate the assembly of cell rafts [20], and FlhE, a small protein thought to wet or to lubricate the surface [21].

It has been estimated that swimming cells use about 2% of their biosynthetic energy output to synthesize flagella and to swim [17]. Energy for rotation is derived directly from the cell's transmembrane proton potential, not indirectly via ATP [17]. An additional genetic cost is incurred in the form of the more than 50 genes (close to 1% of the *S. typhimurium* genome) that are needed for the assembly of flagella and for regulating expression of flagellar genes. These genes happen to be clustered within three loci around the *S. typhimurium* chromosome, and their approximate functions have been established, as well as their regulation.

Flagellar Differentiation

As just described, the swarming of *Salmonella* involves elongation of the cells and an increase in the number of flagella. Even though these changes reverse spontaneously when the bacteria are returned to a liquid medium and begin to grow, the changes can be seen as cell differentiation for the purpose of swarming. How this differentiation might be induced is suggested by a genetic dissection of *Vibrio parahaemolyticus*, a marine bacterium that happens to be a human pathogen. Although polar flagella for swimming are produced continuously during growth in liquid medium, lateral flagella (for swarming) are produced when *V. parahaemolyticus* is grown on solid media and rotation of the polar flagellum is slowed [22]. Then, by suppression of cell division, swarmer cells differentiate [23]. The result is elongated swarmer cells, 5 to 20 times the length of swimmer cells, bearing many lateral flagella that contain a new flagellin. *V. parahaemolyticus* has two circular chromosomes with a total of 5.2 Mb of DNA [4], and more than 38 lateral flagellar genes are encoded on the 1.9 Mb chromosome 2 [24]. Expression of these genes is regulated by the *cheYZABW* or *cheVR* genes that reside on the 3.3 Mb chromosome 1 [4]. Because these genes govern swimming chemotaxis, it appears that 'chemotaxis' genes were co-opted to regulate expression of the lateral flagella [4].

When the flagella differentiate, many new chemoreceptors are also expressed. Thirteen receptors (ordinarily there are four) are encoded on chromosome 1 and sixteen on chromosome 2. The new chemoreceptors were found at both cell poles and at intervals along

the body of the differentiated swarmer cells [4]. Perhaps the sub-polar chemoreceptors are located at the incipient poles of the elongated swarmer cell considered a cryptically multicellular filament. So considered, the data suggest a bipolar localization of the chemoreceptors, although the cells bear polar flagella at one pole. Although the genes that encode lateral flagella specify peritrichous, proton-driven flagella for swarming, their regulation more closely resembles the *V. parahaemolyticus* polar system than the regulatory hierarchy of the peritrichous, proton-driven flagella employed for swimming and swarming by *E. coli*, *S. typhimurium*, or *Pr. mirabilis* that was described above. For instance, *V. parahaemolyticus* employs no Flh DC homolog of *Pr. mirabilis* [4].

Although flagellar differentiation can be understood on the genetic level to some extent, other issues concerning the mechanics and the regulation of swarming with flagella remain to be clarified. First, *V. parahaemolyticus* uses more genes to swarm than *E. coli*, *S. typhimurium*, or *Pr. mirabilis* uses to swim. Why? Second, how can the simultaneous rotation of many flagella on the same cell lead that cell to move over the surface of moist agar? There is evidence suggesting that the peritrichous flagella can bundle with each other [4], making it likely that all the flagella on a cell rotate in the same sense. Bundling may be a hydrodynamic consequence of co-rotating helical flagella [25]. But how is co-rotation ensured and how are all the peritrichous flagella switched simultaneously? Third, how can the rotation of flagella on adjacent cells be coordinated in order that a raft of several cells can move as a unit? In other words, what prevents flagella on adjacent cells from becoming knotted rather than smoothly bundled? How might the chemosensory network bring about proper coordination? Fourth, non-chemotactic mutants of *Pr. mirabilis* still swarm even though the chemosensory genes are required for swarming. Also, many non-swarming mutants of *Pr. mirabilis* are still chemotactically attracted by amino acids and peptides [26]. These observations imply differences between the chemotaxis regulatory network and the swarming regulatory network. Moreover, only two of the four chemoreceptors in *E. coli* are necessary to support swarming, but the two need not be able to sense their most powerful attractant [27]. Fifth, what roles do 29 different chemosensory MCPs play in the swarming of *V. parahaemolyticus*? Might they have a role in controlling gene expression rather than in detecting attractants or repellants? Sixth, why are long, peritrichously flagellated cells differentiated for the purpose of swarming? Is it because by elongating, they become more flexible than single cells? And seventh, what is the relationship between swarming and growth? I would like to suggest that answers to some of these questions may be found in the swarming of bacteria that lack flagella.

Swarming without Flagella

Pseudomonas aeruginosa strain PAO1 uses both flagella and type IV pili [28] to swarm. It retains part of its capacity to swarm after it has lost its flagella [29,30]. This shows that PAO1 swarming can be driven by type IV pili. Perhaps what Henrichsen designated 'twitching motility' and 'gliding motility' in 1972 are, in fact, swarming that is propelled by type IV pili [31]. In hindsight, it is ironic that the scholarly Henrichsen perceived the connection between cells gliding and twitching over surfaces by means of type IV pili correctly [8]. But he seems to have missed the connection between gliding, twitching and swarming because too little was known of their mechanism in 1972. Today it seems likely that the spread of *Neisseria meningitides* and *N. gonorrhoea* in diseased tissues depends on their type IV pili [32,33]. *Neisseria* are not known to have flagella. For these reasons I believe that swarming in general should be understood to include those instances of efficient surface-spreading, described in their own literature as swarming. I suggest that swarming be defined simply as "the process in which motile organisms actively spread on the surface of a suitably moist solid medium" [34].

To appreciate the value of including non-flagellates, let's return to *C. hutchinsonii*, the cellulolytic gliding bacterium described in the Introduction. No differentiation is necessary for *C. hutchinsonii* to move over the cellulose it is consuming. The correlation between the movement of *C. hutchinsonii* and growth is evident to the naked eye, and *C. hutchinsonii* movements over cellulose seem to facilitate its consumption. Bacteriodetes, in general, are plentiful in soil that is rich in particulate organic material; some of them are capable of digesting one of a number of polymers: agar, cellulose, chitin, pectin, keratin, or proteins [35]. I suggest that such concerted movement of many cells be considered swarming. Evidently, the capacity to swarm depends less on the particular engine that is employed to propel cells and more on a behavioral algorithm that enhances the flow of cells away from the center of the swarm to the edge. So understood, swarming is a complex behavioral trait that can be found in a wide variety of Gram-negative and Gram-positive species that happen to have surface motility.

Swarming Myxobacteria

The swarming of several different species of myxobacteria has been documented in a remarkable set of time-lapse movies [36–43]. The movies illustrate an ability to organize swarms that spread rapidly over surfaces and proceed to show the way that elementary swarming behavior is regulated to build fruiting bodies and to differentiate spores within those bodies. In short, the movies show how one program for swarming can be pre-empted for different programs of multicellular development. Some of the regulation of the myxobacterium, *Myxococcus xanthus,* has been studied in molecular detail. *M. xanthus* cells average 7 microns in length and about 0.5 microns in width. They are long, thin, and flexibile; mechanical flexibility should aid their swarming [44].

Pulling

Several long, thin fibers, called type IV pili, extend from the leading end of each cell [45]. Pilus tips specifically attach to fibrils on a group of cells ahead [46,47]. It appears that once an attachment has been made that can withstand 100 pN of pulling force [48], the pilus retracts from its seat in the cytoplasmic

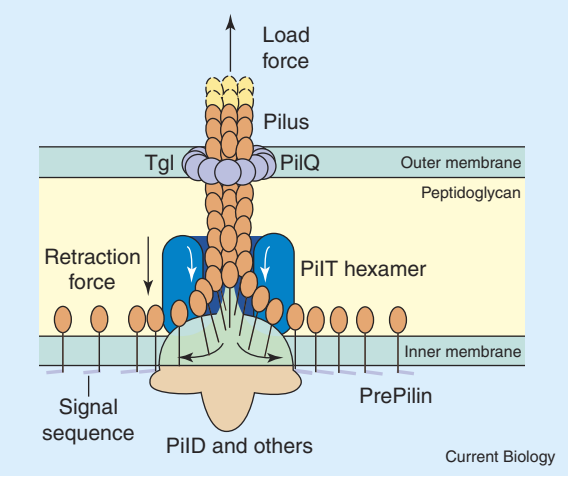

Figure 2. Type IV pilus engine.

TgI is an outer membrane lipoprotein. PilQ is the secretin protein. PilT, a AAA ATPase, retracts the pilus fiber. A movie illustrating pilus retraction (and extension) can be found in [49]. (See text for details.)

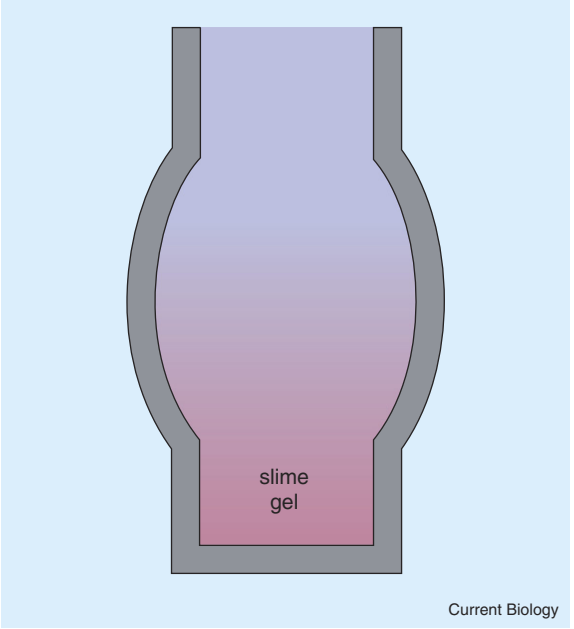

Figure 3. Gel expansion within a nozzle provides a motive force.

A repeat unit of polysaccharide slime is synthesized at the inner surface of the cytoplasmic membrane shown at the bottom of the diagram. The repeat unit is partially dried when it 'flips' through the cytoplasmic membrane to its outer surface, shown in red at the bottom of the nozzle. The nozzle chamber fills with water from the environment, represented by the blue color. Binding water, the polysaccharide gel swells, represented by the intermediate color of the nozzle chamber. Because the walls of the nozzle are stiff, the gel pushes out the open end at top of the diagram as the gel swells. The mechanism is based on [55,56,89].

membrane [49]. Retraction pulls the piliated cell forward; it is observed (my unpublished observations) that the group to which attachment has been made moves but slightly, probably due to its greater size and greater adhesion to the surface.

Type IV pili are found only at one pole of *M. xanthus* [45]. As a molecular machine, the pilus engine is built from more than 15 different proteins. Those proteins are found in the cell's cytoplasmic membrane, across the periplasm where the machine is most likely anchored to the peptidoglycan, and finally out of the cell by crossing the outer membrane [31]. The structure is illustrated in Figure 2. After removal of the signal sequence by PilD, many thousands of pilA monomers are polymerized to form the helical pilus fiber that often grows to several times the length of a cell [50]. The pilus fiber passes through a sealed bushing in the outer membrane, composed of PilQ protein [51]. TgI, a 17 kDa lipoprotein, is necessary to assemble PilQ monomers into the bushing-like gated channel in the outer membrane [52]. The structure of PilT, a AAA ATPase, has recently been solved, indicating its mode of action is the motor for pilus retraction [53]. Retracted pilin monomers are stored in the cytoplasmic membrane for reuse [49]. *M. xanthus* PilT closely resembles that of *Neisseria gonorrhoea* and can develop more than 110 pN of tension [54]. Considerable energy is required both for extending — which is catalyzed by PilB, another AAA ATPase, in the vicinity of PilT — and for retracting a pilus fiber [48]. The capacity to generate so large a force suggests that the pilus engine is rigidly attached to the peptidoglycan. Relative to the gliding force generated, the energy requirements are comparable to the requirements for rotating flagella discussed above, although they use ATP.

Pushing

The trailing end of an *M. xanthus* cell has many slime secretion engines capable of pushing it forward. Electron microscopy reveals that filaments of slime emerge from a cell end [55], and light microscopy shows that several filaments merge into a single filament of cell width [56]. It is striking that both electron and light microscopy show that a united filament emerges only from one end of a cell; the opposite end lacks a filament. However, several hundred tiny, thick-walled pores, believed to be secretory nozzles, are found at both cell ends in *M. xanthus*, suggesting that nozzles at one cell end may be secreting, while those at the other end of the same cell may not. Biochemical genetic experiments [56] indicate that the slime ribbons visible by electron and by light microscopy comprise a hydro-gel. The gel formed when the repeat unit slime polysaccharide, whose synthesis resembles the synthesis of *E. coli* capsular polysaccharide, is secreted through a Wza-like translocon [57], and absorbed water flowed into the nozzle from the environment. This is diagrammed in Figure 3.

The hydro-gel left behind on the agar by a moving cell forms a slime trail. Movement coordinated trail growth is illustrated in Figure 4. Many gliders have been observed to create trails on agar [58]. When another cell encounters a trail, it usually turns through the acute angle of intersection to follow the trail [59]. The new gel fuses with the trail gel, which allows the newly secreted slime to push against the polysaccharide chains of the trail. In this way, the old and new

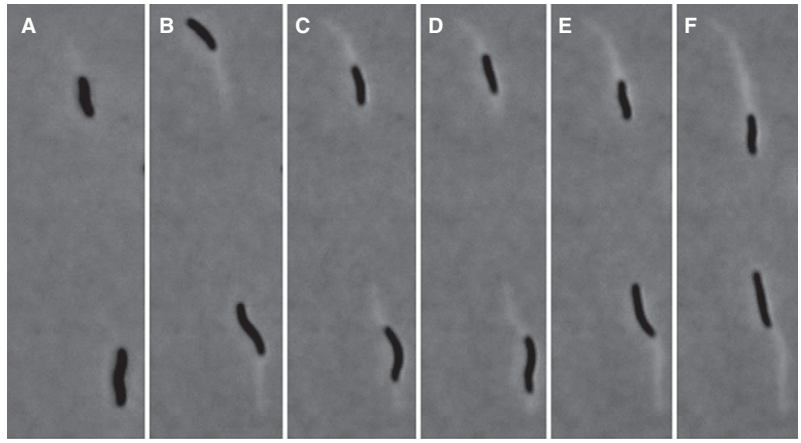

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Figure 4. Laying down a slime trail, reversing, and following the trail.

Two A^+S^+ cells gliding on an agarose gel. Six frames were selected from a time-lapse movie taken by Lars Jelsbak using a Nikon 40x phase contrast objective. The slime trails elongate progressively as the cells move over the surface. The following observations were made stepping frame by frame through all 60 frames. Frame (A) is the start, there are no trails. In frame (B) both cells have moved upward, leaving a trail behind them. In (C) both cells have moved downward following their own trail. In (D) the lower cell moves down, still following its own trail. In (E) the upper cell moves down on its own trail, while the lower cell moves up on its trail. Evidently, the two cells do not reverse at the same time, each has its own clock. In (F) both cells move farther along their own trail.

polysaccharide chains are oriented along the same axis. Trails can be followed in either direction, and one often sees a cell reversing to glide on a trail it just laid down in the opposite direction, as shown in Figure 4. These mechanical details suggest that the following of slime trails is closely related to elasticotaxis, a phenomenon by which *M. xanthus* cells preferentially glide in the direction of the lines of elastic stress created by compressing agar [7], and both have the same bidirectionality. Lines of stress correspond to the average orientation of the agarose chains that is produced by the compression of an agar gel. Thus trail following and elasticotaxis are consequences of the polar orientation of slime secretion, and they are found to be dependent on the slime secretion engine as well as independent of the type IV pili [56].

Like flagellar rotation and the pulling of type IV pili, the process of slime secretion is expected to consume large amounts of ATP [48]. Moreover, *M. xanthus* does not metabolize glucose or other hexoses, and is believed to synthesize monomer units for all its polysaccharides by gluconeogenesis [60]. With respect to the mechanics of gliding, it is important that the slime engine is unidirectional like the pilus engine. The ability of cells to move demands that the slime engine always be located at the back of the cell, while the pilus engine always be located at the front. This proposition relates to the observation that the two engines always cooperate with each other.

Cooperation between Engines and between Cells

Figure 5 displays several basic qualities of *M. xanthus* swarming. The left panel shows that swarms expand at a constant rate. Generally the rate remains constant until the edge of the swarm reaches the edge of the agar slab on which the swarm is expanding. The panel also shows that 90% of the expansion rate is due to cell movement, and only 10% results from the concurrent growth. Nevertheless, growth is necessary to give cell flow its radial direction (Figure 1). Evident in the panel on the right, the ultimate rate of swarm expansion increases monotonically with the density of cells used to initiate the swarm, expressing the cooperative nature of swarming. Finally, the expansion rates for wild type (A^+S^+) are 50% greater than the sum of the rates for two mutants that lack either one (A^-S^+) or the other (A^+S^-) gliding engine. The two engines always support each other; no opposition is evident between engines. As one engine is built to pull, while the other is built to push, synergism expresses the intrinsic polarity of *M. xanthus* cells. Their structure insists that the pulling pili are always at their leading pole and the pushing slime nozzles at their trailing pole. The challenge is to identify the structural elements that are critical. An effort to do so is described next.

Reversal and Swarming

To summarize the problem, the location and the functional polarity of both engines seem linked to *M. xanthus* cell structure. Moreover, there are many copies of each engine, perhaps 300 slime nozzles and a dozen or so pili located at each cell end, and each engine is a multi-protein machine. How then are we to understand the ability of many hundreds of engine proteins to change their structure from active to inactive, or the reverse, simultaneously and appropriately in order to reverse their gliding direction? It has long been known that growing *M. xanthus* cells reverse their gliding direction at roughly 8 minute intervals, during growth and fruiting body development [61–63]. Repeated slow viewing of Reichenbach's movies have convinced me that cells reverse simply by stopping and within about a minute moving off in the opposite direction.

The simplicity of the macroscopic reversal process seems at odds with the microscopic complexity of the protein structural changes that occur. And yet simple transitions have been confirmed in recent movies [64,65], and by repeated viewing of the movie from which a few frames were presented in Figure 4. One clue as to how two inherently unidirectional and multi-protein engines reverse is suggested by the finding of two different localization patterns for groups of Pil proteins. Using PilQ-specific antibodies, the protein

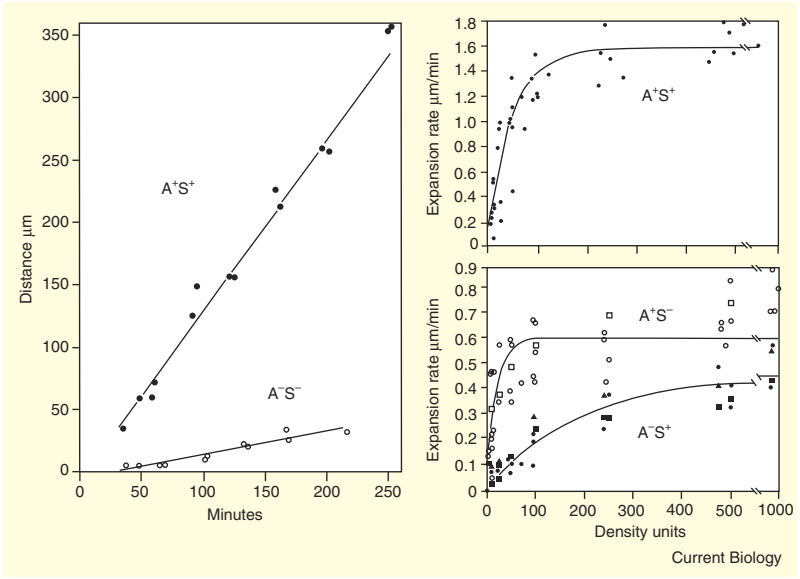

Figure 5. General features of *M. xanthus* swarms.

A^+ indicates a normal slime secretion engine. A^- indicates a defective slime secretion engine. S^+ indicates a normal pilus engine. S^- indicates a defective pilus engine. (Data from [90], which also contains a description of the methods used to quantify swarm expansion.)

is found localized to discrete patches at both cell poles [52]. PilQ exemplifies the bipolar pattern. In the same set of experiments, now using Tgl-specific antibody, Tgl was localized to a patch only at one pole [52]. Moreover, pili were found at only one pole [45]. Tgl and pili exemplify the unipolar pattern, and Tgl is expected to be localized to the leading pole, which is piliated. Neither the patching of PilQ, nor its bipolarity depend on the presence of Tgl because both were found in a *tgl* deletion mutant strain [52].

These data suggest that the PilQ patches at both poles are resting on top of an assembly of multiple Pil proteins that will be called the pilus basal body. The basal body would include all the Pil proteins except PilA (the pilin) and Tgl (the assembly factor); it would include PilB, PilM, PilN, PilO, and PilT proteins [52]. These proteins are also found in the inner membrane and periplasm in *N. gonorrhoeae* [66]. The *Ps. aeruginosa* inner membrane pilus proteins PilB and PilT have been shown to be bipolarly localized and its pili are strictly unipolar [67]. The observed bipolarity of PilQ patches implies that the basal body is also found at the non-piliated end. Accordingly, the PilQ patch at the back end of the cell has neither Tgl nor a pilus fiber and its PilQ is not assembled into a gated channel. It is proposed that, when PilA accumulates in the inner membrane at the back end of the cell, and when Tgl accumulates in the outer membrane there, then PilQ changes from a cluster of monomers into a detergent-resistant sealed bushing [52]. Then the pilus can slide in or out through the assembled PilQ without loss of vital periplasmic proteins.

A similar scheme could explain the reversal of the engines that secrete slime. These engines also have unipolar and bipolar groups of proteins. CglB is an outer membrane lipoprotein [68,69] that has been shown to be required for unipolar slime secretion [56]. CglB is efficiently transferred from cell to cell by stimulation, like Tgl [70], consistent with a unipolar localization in the outer membrane. Slime secretion nozzles, visible by electron microscopy, are bipolar: they are always seen at both cell ends [55]. The nozzles, like the pilus basal body, are expected to include the proteins that synthesize, export, and regulate slime production. It was suggested above that the nozzle proteins have a structure related to the protein complex responsible for the biosynthesis of K30 capsular polysaccharide in *E. coli* [71,72]. The complex includes a Wza-like translocon that is shaped like a vase or nozzle [57]. However, the aperture of Wza in the outer membrane is 17 Å in diameter, while the inner diameter of the slime nozzles is 65 Å [55]. A larger orifice would permit the simultaneous secretion of several polysaccharide chains each having the 17 Å diameter of a capsular polysaccharide, enabling secretion of a wider hydro-gel ribbon [55]. On the negative side, Mignot *et al.* [65] claimed that cells are not propelled by secreting slime as they observed, in moving cells, focal adhesions that remain fixed to the agar support. They did not, however, examine slime secretion, and none of their observations rule propulsion out. Those authors merely expressed a preference.

The many *mglA* mutants, all of which fail to swarm [73], offer a second important clue to the mechanism of reversal. MglA mutants are unique among non-swarming mutants of *M. xanthus* in that they can arise from A^+S^+ strains in one mutational step (AS symbols defined in the Figure 5 legend); all other non-swarming mutants, recognized by their sharp colony edges, require one mutation to inactivate the pilus engine and another mutation to cause bipolar slime secretion [74]. As mentioned above, motile strains secrete a ribbon of slime only from one end at any instant of time. But *mglA* mutants are found to secrete slime simultaneously from both ends [56]. They fail to swarm because by trying to move in both directions simultaneously, they cannot move significantly in either [75,76]. Bipolar slime secretion strongly suggests that the role of MglA is to switch the engines from one end to the other. Lacking the switch, the cell accumulates slime engines at both poles, as observed [56].

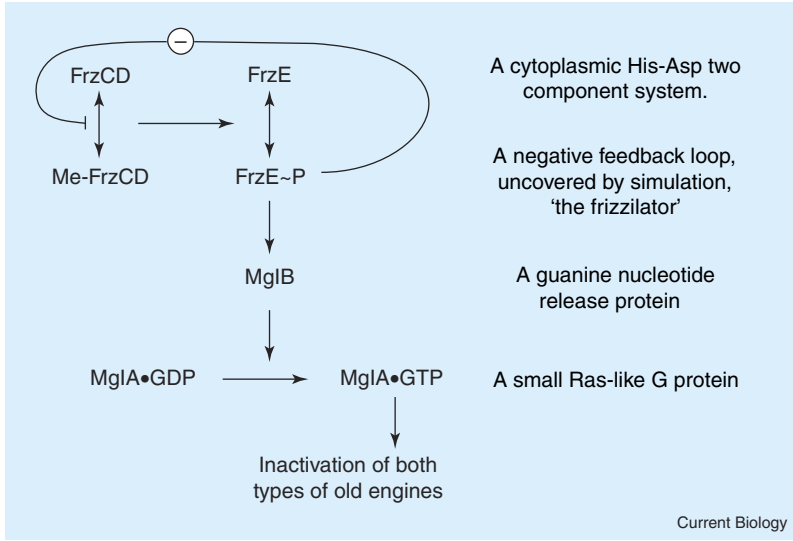

Figure 6. Model of the reversal clock that is based on the 'frizzilator' [80] and a G-protein switch.

Whether pilus engines are also present at both poles has not yet been checked for technical reasons. MglA is a small G protein that would have GDP-bound and GTP-bound forms, and is consequently the prime candidate for the reversal switch; its role would be to initiate loss of the old engines.

That the frizzy chemosensory proteins regulate the frequency of reversal has been known for 20 years [61]. A molecular circuit having these properties is represented in Figure 6. This model for engine switching uses a clock to trigger the exchange of both slime secretion and pilus engines between poles. The switch includes a methyl-accepting chemosensory protein, FrzCD, which recalls the use of MCPs in flagellar swarming. Blackhart and Zusman [61] discovered that FrzCD controls the frequency of gliding reversals in *M. xanthus*, both upward and downward. FrzCD differs in important ways from the chemotactic MCPs of *E. coli* and *Salmonella*: FrzCD is a cytoplasmic protein, and it lacks transmembrane and receptor domains [61,77], so it is unlikely to be a membrane receptor like the chemotactic MCPs of *E. coli*, *S. typhimurium* and their close relatives [78]. The histidine protein kinase of this two-component system in *M. xanthus* is FrzE, an autophosphorylating histidine protein kinase [79]. Because wild type cells reverse every 8 minutes (in traveling waves), and because a *frzE* null mutant almost never reverses, FrzE~P is the major candidate for the signal to reverse polarity in *M. xanthus*.

When Igoshin *et al.* [80] succeeded in simulating traveling waves mathematically, they found that the levels of Me-FrzCD and FrzE~P oscillate out of phase with each other. To explain these oscillations they found it necessary to postulate a negative feedback from FrzE~P back onto the methylation of FrzCD, which creates a 'frizzilator' (Figure 6). This particular loop was the simplest way to obtain oscillations like those observed. The simulation did not indicate whether the feedback inhibits methylation or stimulates demethylation, and distinguishing those will require some biochemistry. Also for simplicity, the model places MglA, a small Ras-like G protein, and its guanine nucleotide release protein, MglB [81,82], just downstream of FrzE~P. A biochemical precedent for this mechanism is the activation of the flagellar switch complex by CheY~P, discussed above in connection with flagellar swarming. MglA-GTP would then select the cell pole that should lose its type IV pili, probably by losing Tgl, and the pole that should lose slime secretion, perhaps by gaining CglB. Genetic epistasis tests confirm that MglA controls both engines [76]. It is suggested that the reversal clock consists of the frizzilator plus MglA and MglB, and that it switches periodically. The regulatory circuit shown in Figure 6 is supported by mathematical simulation [80]; it is also supported by mutational studies of the Frz and MglA proteins that have been reviewed [83].

Because the unipolar Tgl and CglB protein disappear from their old poles, they may be degraded; the genome includes numerous genes for candidate proteases, but they remain to be screened. Alternatively, pole-to-pole migration of these outer membrane lipoproteins, as described for AglZ [65], has not been ruled out. In any case, without Tgl, the PilQ secretin would disassemble because the monomers are not covalently bonded together. PilQ is observed to remain as a condensed patch in the outer membrane [52] and expected to remain attached to the top of the pilus basal body. Extending from PilQ in the outer membrane to PilT in the inner membrane, as in *Ps. aeruginosa* [67], the basal body must pass through the peptidoglycan meshwork in the periplasm. As a consequence, the disassembled patch of PilQ would be expected to retain a polar localization, as is observed.

Having disassembled both engines at their old poles, how might the proper new engines assemble at the opposite poles? Figure 7 illustrates the fate of both *M. xanthus* engines when a cell grows and divides. Consider the two new ends created by the division septum. Automatically, each daughter receives only one of the two engines at its new pole. Many examples in Reichenbach's and later movies show that both daughter cells are motile at birth; no gestation is evident. Each daughter always assembles

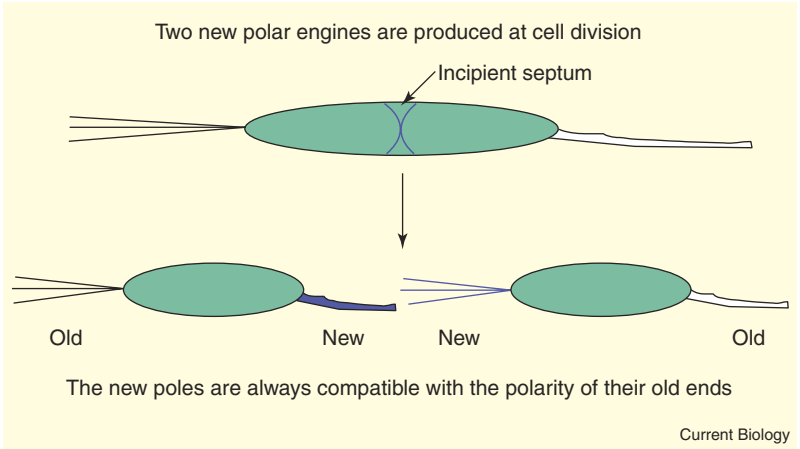

Figure 7. Polarity is conserved throughout growth and cell division.
Old engines are colored black; new engines, blue.

the proper engine at the proper pole for purposes of engine coordination, as Figure 7 illustrates. This fact of myxobacterial life suggests that the peptidoglycan, and possibly other cytoskeletal elements, constitute a polarized template specifying that different engines be active at the opposite poles.

Rudiments of both engines are always present at both ends, the pilus basal bodies and the nozzles. But only two possibilities are allowed at a new end: either active pili with inactive slime nozzles (nozzles not extruding slime), or active nozzles that are extruding slime with inactive pili (pilus basal bodies without pilus fibers or Tgl). *M. xanthus* cell division is symmetric, and it differs from the asymmetric divisions of *Caulobacter crescentus* and sporulating *B. subtilis* [84]. Moreover, the polarized template interacts with the reversal switch in such a way that every cell end has many copies of its proper engine in their working state and many copies of the other engine, none of which are working. All the engines switch together; it is all or none. It seems likely that template and switch evolved together in order that *M. xanthus* be able to swarm.

Summary and Concluding Observations

Bacteria that have the ability to translocate over surfaces appear to have evolved the ability to swarm. Translocation by rotating flagella, by pulling with type IV pili, pushing with slime secreted from the rear, or some other means such as those employed by *F. johnsonae* [11] have all been used for swarming. As a consequence, these bacteria are able to continue to grow after their cells have begun to compete with each other for access to nutrient. In liquid suspension, these bacteria would eventually be forced to balance growth and death and to enter a stationary phase. By translocating to the edge of the swarm, they are able to maintain rapid growth. The coupling between movement and growth is evident in *Pr. mirabilis,* which alternates growing and spreading phases [85]. It is also evident in the ascending colonization of the urinary tract by *Pr. mirabilis* [86]. Similar coupling is evident in the spreading of *Neisseria* into a tissue it is infecting [33]. The inevitable conflicts between cells for real estate are minimized by enhancing cell flow and avoiding creation of traffic jams.

When a motile bacterium begins to evolve swarming behavior, it has a regulatory system built-in, such as a chemosensory system for flagella, one or more two-component systems for type IV pili, and a polysaccharide biosynthetic control system for slime secretion. Each species would then have had to adapt its built-in regulatory system to handle swarming. For example, *M. xanthus* seems to have reduced an MCP from a membrane protein to a cytoplasmic protein, and to have added a negative feedback to the Frz two-component system to create a reversal clock. We now know that the clock is essential for swarming. Flagellated bacteria have modified their (built-in) chemosensory system to regulate flagellar gene expression rather than performing chemotaxis [4].

Movement behaviors that facilitate swarming can be identified among the properties shared by swarmers with different engines. There is a net radial outflow of cells from the swarm center that is driven by growth and cell division in the center of the swarm shown in Figure 1C. This follows from several observations. Swarms have radial symmetry (Figure 1). When *M. xanthus* recognizes an amino acid shortage, the stringent response stops growth, and cells immediately stop outward swarming [63,87]. The observation that Cytophaga and Flexibacteria swarm as they feed on insoluble organic materials makes a similar point.

Considering the correlation between growth and flow, one could assert that the aim of swarming behavior is to enhance cell flow. We are painfully aware of the problems associated with flow at high cell density (the condition faced by swarm cells) as we merge our motor car into freeway traffic. When the density of cars is high, traffic is stop and go, and travel becomes inefficient. But traffic can flow smoothly even at high density, if each car travels at the same average speed as the car in the lane ahead. Swarming bacteria match their speed and achieve smooth flow among many thousands of individual cells at very high density.

Another enhancer of cell flow that is found with all types of engine(s) is that swarm cells are all long and they are flexible. Flexible cells can glide over a surface of arbitrary shape. Elongated cells, gliding in the direction of their long axis can also slip past one another to maintain the cell flow.

Having observed many swarms of *M. xanthus*, I can affirm that swarm cells tend to move continuously, and to reverse periodicially. Continuous movement maintains the flow. Reversals seems to prevent traffic jams that would otherwise form. Imagine the Place de la Concorde in Paris at 4pm. If cells stalled after colliding with other cells, and that is exactly what they do, reversal allows them to recover. Considering the problem of jams, it is obviously an advantage to have a pushing engine on a flexible cell with a rounded tip. Myxobacteria combine these two properties. The combination helps resolve end to side collisions by the bending and turning of the colliding cell. While bending cannot resolve collisions that happen to be at right angles, reversal can.

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